

Promotion of Gastrointestinal Tract Tumors in Animals: Dietary Factors

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The biological mode of action of tumor promoters, exemplified by the phorbol esters, is a subject of intensive study in a number of laboratories. A few investigators have recently begun to examine the role of dietary nutrients in tumor promotion, but the available data are sparse and interpretation difficult. A few examples are provided to indicate that some nutrients may be important in the promotion of cancer. However, the fine dividing line between effects on initiation or on promotion, so clearly shown in the mouse two-stage skin cancer model, is not so clear as yet in models used for studies in nutritional carcinogenesis. The animal models for these studies have been primarily rats, mice and hamsters. These have shown that nutrients which appear to have promotion activity are zinc deficiency and 13-*cis*-retinoic acid for the esophagus; vitamin A deficiency and lipotrope deficiency for the forestomach, unsaturated fat and vitamin A deficiency for liver and colon, lipotrope deficiency for the liver; selenium for the liver. It is probably more correct at this early stage of investigation to consider the effects of nutrients acting either during the time of exposure to the carcinogen, or, after such exposure and when no detectable carcinogen is found in the animals tissues, rather than as promoters in the strict sense.

Introduction

It is becoming increasingly clear that the carcinogenic process in both humans and experimental animals is probably a multistep process which occurs over long periods of time. Furthermore, many factors appear to interact in the evolution of a malignant tumor from an initiated cell. When a group of normal cells is exposed to a carcinogen, neoplasia does not happen immediately. There is a lag time, ranging from months in animals to decades in humans. Following initiation by a carcinogen, a step-wise change in properties of the cell occurs to include hyperplasia, metaplasia, benign neoplasia and finally malignant neoplasia. It is the malignant tumor that is of greatest significance to the host, because benign tumors can be excised and rarely if ever create a problem, aside from mechanical interference with normal function of the organ in which it is located.

It is essential that we understand factors and events taking place during the transition of normal to malignant cells if means for interrupting these changes are to be identified. In some cases the early stages of tumor formation are reversible and furthermore some tumors do in fact regress; in the

later stages of tumorigenesis the process is less easily reversed.

The concept of at least two stages in the development of a tumor (initiation and promotion) has been generally accepted by oncologists. Thus attempts have been made to identify initiating agents and promoting agents and to further define their modes of action (1-6).

A major advance in knowledge about tumor promotion was the isolation and identification of the phorbol esters which are active principles of croton oil, the most classical tumor promoter. Since the phorbol esters have been identified and made available for experimentation, they have been the subject of intensive study in a number of laboratories, relative to the biochemical and biological effects of these chemicals and related materials. However, not all of the biological effects are necessarily those associated with tumor promotion, but they may aid in the elucidation of the mode of action of tumor promoters and are usually discussed within the same context as promoters. The term, tumor promoter, is often used interchangeably with cocarcinogen, synergist, or accelerating and enhancing agent. There is, however, a distinction in the method of biological testing for tumor promoters and for cocarcinogens.

The majority of studies involving initiation and promotion have been done by using the mouse skin

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model for two-stage carcinogenesis which involves an initiating agent and a promoting agent. By definition, an initiating agent is an agent which results in benign and malignant tumors when applied on mouse skin in a single dose, followed by repeated applications of a promoting agent. When the initiating agent is given alone it does not result in the induction of tumors because of the dose that is used. However many of the agents that are used as initiators in two-stage carcinogenesis, such as the aromatic hydrocarbons, benzo(a)pyrene and 7-12-dimethylbenzanthracene, are carcinogenic. Furthermore, some noncarcinogenic initiating agents for mouse skin are also known, as shown in Table 1. In addition, not all chemical carcinogens are initiating agents. They are usually carcinogens which have to be activated *in vivo* to become the carcinogenic intermediates.

The definition of a promoting agent may differ from one investigator to another, but in general it is an agent which, when applied repeatedly after a single dose of an initiating agent, results in benign and malignant tumors. Furthermore, all known promoting agents have in addition weak tumorigenic activity. Initiators and promoters are mechanistically and temporally interrelated and cannot be separated. If the sequence of applications is reversed, few if any tumors appear. While the phorbol esters and their derivatives are best known and the most widely studied promoters there are others that are of historical interest including (7), anthralin (8) and dodecane.

The above definitions of initiator and promoter are for operational use only but serve a purpose in the context of this presentation. There are many others which are much more exhaustive and perhaps even more accurate (9).

An additional definition that may be useful is that of a cocarcinogen. In studies dealing with cocarcinogenesis, two agents are administered simulta-

neously, usually in repeated applications or exposures, and the combination of the two results in higher tumor incidences than either agent alone. For this reason, there is an operational difference and probably a real difference in mode of action between cocarcinogens and tumor promoters since not all cocarcinogens are tumor promoters nor are all tumor promoters cocarcinogens (6). Table 2 compares the activities of tumor-promoting and cocarcinogenic agents.

Table 3 lists the characteristics of stages of initiation and promotion in skin carcinogenesis as elucidated by Pitot (9). This is not to imply, however, that these same characteristics may apply to carcinogenesis in other organs and tissues.

Environmental agents including diet and nutrition can modulate the effects of promoting agents. As early as 1944, Tannenbaum demonstrated an effect of nutritional tumor promotion (10). Later, others showed effects on tumor promotion by nutritional factors by varying the quality and quantity of

Table 2. Comparison of tumor-promoting and cocarcinogenic activities.^a

Compound	Tumor-promoting activity	Cocarcinogenic activity
Phorbol myristate acetate	+	+
Anthralin	+	+
<i>n</i> -Dodecane	+	+
Linalyl oleate	-	+
Phenol	+	-
Catechol	-	+
Resorcinol	-	-
Hydroquinone	-	-
Eugenol	-	-
Pyrogallol	-	+
Pyrene	-	+
Fluoranthene	-	+
Benzo(e)pyrene	?	+

^aData of VanDuuren (6).

Table 3. Characteristics of stages of initiation and promotion in skin carcinogenesis.^a

Initiation	Promotion
Irreversible, with "memory"	Reversible, at least in early stages
Initiated cells and immediate progeny not usually identifiable	Promoted neoplasm seen grossly
"Pure" initiation (incomplete carcinogen) causes irreversible change but no neoplasm unless promoter applied	Promoting agents not carcinogenic but may promote fortuitously initiated cells (i.e., background)
Dependent on cell cycle and, for many chemicals, on the metabolism of the cell	Modulated by diet, hormonal, environmental and related factors

^aData of Pitot (9).

Table 1. Initiating agents which are not carcinogenic on mouse skin.^a

Compound
Dibenz(a,c)anthracene
Chrysene ^b
Benz(a)anthracene ^b
Urethane ^c
Triethylenemelamine
1,4-Dimethanesulfonoxo-2-butyne
Epichlorohydrin ^d
Chloromethyl methyl ether ^d

^aData of VanDuuren (6).

^bThese are considered borderline carcinogens by some.

^cCauses lung tumors in mice, lymphoma in rats.

^dInduces sarcomas in mice by subcutaneous injection.

nutritional components fed to the animals during carcinogenesis (11-13). Sporn (14) and others have shown that several types of neoplasms can be prevented by the use of retinoids.

In regard to promoters it should be pointed out that initiating agents are mutagenic because they bind covalently to DNA. Phorbol ester, for example, a promoting agent does not bind covalently to DNA and is not mutagenic. However, continued long-term painting of phorbol ester on mouse skin may result in a small number of tumors. These observations point out the reason that the Ames assay or other conventional mutagenesis assays will not pick up promoters, and further there is no evidence to date that this class of promoters requires metabolic activation. This is in contrast to most of the initiating agents.

Nutrients and Nutrient Deficits as Promoters of the Carcinogenesis Process

At this point it is not clear as to whether or not nutrients per se or deficiencies of nutrients are acting as promoters or are acting in concert with other factors to enhance or inhibit carcinogenesis. Except for mammary carcinogenesis, where there does seem to be some evidence for tumor promotion, there is little in the literature clearly defining the time at which nutritional deficits or excess exert their effects. For that reason it is not possible to state clearly that we are dealing with a promotional activity in those nutrients that do, in fact, affect the incidence of induced cases—it is possible to define the effects of a nutrient only as they relate to whether they are operating during the time the carcinogen is administered or following the administration of the carcinogen and during the growth stage of tumorigenesis. A part of the problem is the need for repeated application or doses of the carcinogens used in most nutrition studies. Most of the data presented in this paper will attempt to relate the nutrient effects to time: whether during treatment, following treatment, or both. Thus we will not be dealing with the subject of promoters in the strict sense of the definition.

In so far as the gastrointestinal tract is concerned, we will include the liver, although it is being covered in more detail in other presentations at this conference. In addition, we will consider the esophagus, the stomach, the pancreas and the colon.

Esophageal Carcinogenesis

Esophageal cancer offers a unique epidemiologic model for the study of cancer causation and a means

to learn more about initiation or promotion of this unique neoplasm. The 300-fold worldwide variation of this type of tumor provides the opportunity to identify environmental factors associated to increased risk to test these factors in appropriate animal models (15).

Epidemiologic studies have already shown that in areas of low and moderate incidence of the disease, such as the United States and Western Europe, the consumption of high levels of alcohol correlate strongly to increased risk (16). Mossbach and Viddeback (17) have reported that 65% of 84 male esophageal cancer patients were alcoholics, and 21% of them were employed as brewery workers. In other studies it has been shown that the risk of the disease is 17 times greater for alcoholics than nonalcoholics (18). In France and in some parts of Africa, esophageal cancer appears to correlate to the type of alcoholic beverage more than to the amount that is consumed, suggesting some type of contaminant.

In many high incidence areas of the world, such as Iran, parts of Russia, the Transkei of South Africa and parts of China, there is little if any alcohol consumption; other factors must be sought as etiologic agents (19). In a large study in South African esophageal cancer areas, van Rensburg (20) observed that those at high risk consumed dietary staples which result in deficiencies of riboflavin, nicotinic acid, magnesium and zinc. Our own studies have shown that Chinese patients with esophageal cancer in the Hong Kong area have reduced levels of zinc in hair, serum and esophageal tissue, compared to healthy controls or patients with other types of cancer (21). Table 4 indicates the observations in human esophageal cancer patients.

We have followed up on the observations in human esophageal cancer patients using animal models and observed a number of enhancing and probably promoting effects of zinc deficiency (22). A series of studies in rats has clearly shown that zinc deficiency lowers serum, hair and esophageal concentrations of zinc and significantly enhances induction of esophageal tumors by methylbenzyl nitrosamine. Furthermore, the results of recent studies (Table 5) have revealed significant effects of zinc deficiency and alcohol as well as zinc deficiency combined with ethyl alcohol and 13-*cis*-retinoic acid (24). 13-*cis*-retinoic acid combined with alcohol further enhances the effect of zinc deficiency.

Table 6 illustrates clearly the remarkably enhancing effect of zinc deficiency alone but also shows that putting rats back on a control diet after exposure to the carcinogen during zinc deficiency significantly reduced the tumor incidence. This implies that the promotion stage or growth stage of the tumor is affected by zinc deficiency but does not

rule out an effect during the initiation period. These data indicate that zinc deficiency is acting during exposure to the carcinogen (initiation) as well as after the exposure (promotion). The observation of the apparent enhancing effect of 13-*cis*-retinoic acid on esophageal cancer is directly opposed to the usual observation with the retinoids wherein it is beneficial and inhibitory (25). While this observation of an effect on esophageal tumors was not expected, neither is it totally surprising. In other studies using the hamster lung cancer model we found that hypervitaminosis A actually enhanced respiratory

Table 4. Zinc and copper levels in serum and hair of normal humans and those with various disorders.^a

	Zn ^b	Cu ^b
Serum, µg%		
Normal subjects	102.7 ± 18.5(15)	133.4 ± 30.5(15)
Patients with esophageal cancer	78.0 ± 14.9(20)	159.4 ± 44.4(20)
Patients with other types of cancer	114.4 ± 31.8(6)	148.6 ± 102.(6)
Patients with other disorders	96.2 ± 15.0(9)	146.7 ± 39.4(9)
Hair, ppm		
Normal subjects	195.0 ± 29.0(16)	
Patients with esophageal cancer	162.0 ± 33.0(19)	
Patients with other types of cancer	169.0 ± 37.0(4)	
Patients with other disorders	212.0 ± 48.0(8)	

^aData of Lin et al. (21).

^bNumbers in parentheses are number of subjects.

tract tumor formation (26). In this case the excess vitamin A appeared to be enhancing the tumor formation during the promotional stage, since the higher concentrations of vitamin A were administered beginning one week after the last dose of carcinogen (Fig. 1).

Table 5. Effect of zinc deficiency, alcohol and 13-*cis*-retinoic acid on esophageal tumors in rats.^a

Treatment	Tumor induction	
	No.	%
Control diet	0/0	0.0
Control diet + MBN	14/35	40.0
Zinc-deficient diet + MBN	30/34	88.0
Zinc-deficient diet during MBN dosing, then control diet	18/35	51.4
Zinc-deficient diet + MBN + alcohol	25/30	80.3
Zinc-deficient diet + MBN + alcohol + 13- <i>cis</i> -retinoic acid	34/35	97.1

^aData of Schrager and Newberne, (23).

Table 6. Promoting effect of zinc deficiency on esophageal tumors in rats induced by methylbenzyl nitrosamine (MBN).^a

Treatment	Tumor-bearing rats		No. tumors	
	No.	%	Single	Multiple
Control + MBN	5/34	15	3	2
Zn-deficient + MBN	30/34	88	4	26
Control during exposure, then Zn-deficient	21/34	62	9	12

^aRats were given 2.5 mg/kg MBN twice weekly for 3 weeks, total 15 mg/kg body weight.

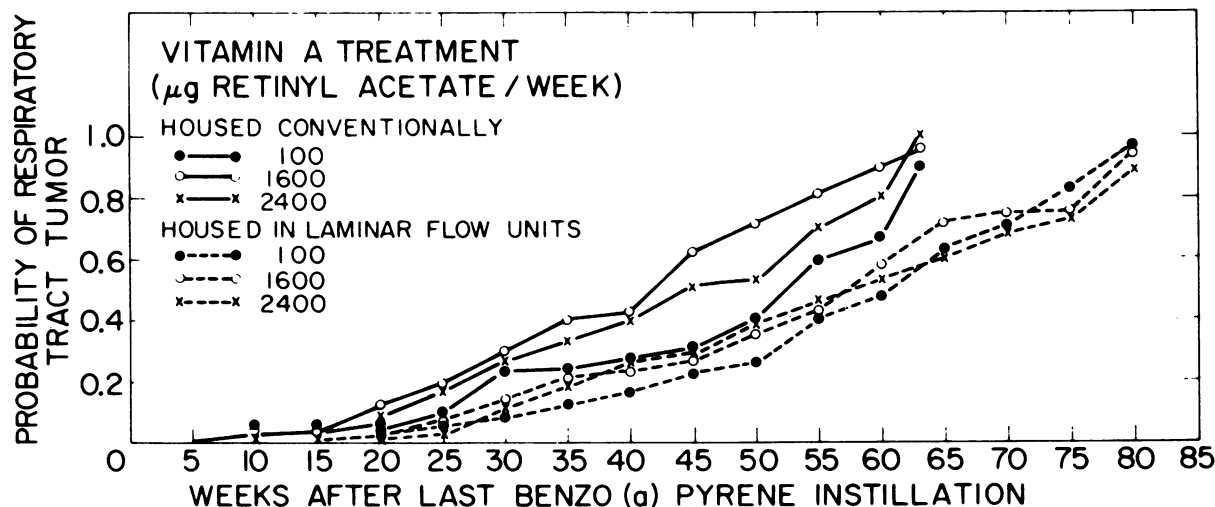


FIGURE 1. Probability of respiratory tract tumors in hamsters given an intratracheal dose of benzo(a)pyrene (BP). The vitamin A was given beginning 1 week after the final BP dose, illustrating a promoting effect.

Gastric Cancer

Gastric cancer was one of the most common neoplastic diseases in the United State at the turn of the century, but the morbidity and mortality from this type neoplasm have decreased steadily in the intervening years. However, in other parts of the world, particularly in the mountain regions of central and western Latin America, northern and eastern Europe, Iceland, and in some parts of Japan, gastric cancer remains one of the more common types of neoplasms. A decreased incidence has recently been observed in some of these areas (27-29).

Etiologic agents for gastric cancer in human populations are not known, but a number of foods and environmental contaminants have been suggested (30, 31).

Migrant studies have suggested that chronic intake of selected dietary factors is associated with high risk for gastric cancer. For example the intake of dried and salted fish or pickled or smoked fish, prevalent in many countries particularly Japan, seem to be important. Local customs and availability of food which sometimes results in decreased intake of fresh fruits and vegetables also seem to be involved. Nitrate, nitrite and nitrosamines have been associated by some with the incidence of gastric tumors in some geographic areas. However, despite all of the epidemiologic and control laboratory experimental work that has been done the causative agent(s) in human gastric cancer remain elusive.

With the advent of an animal model for glandular gastric cancer, numerous investigations have been conducted. The discovery that nitrosourea compounds would produce antecedent lesions as well as gastric cancer has considerably advanced the efforts toward revealing the cause(s) of gastric carcinoma. A number of individuals have shown that an aston-

ishing number of substrates, including dietary amines, can be nitrosated to carcinogenic nitrosamines and nitrosamides in the stomach (32-34).

Weisburger et al. (35) have produced some interesting results with nitrate, nitrite and food extracts, particularly those from fish, which clearly support the results of others. These studies point out (Table 7) that substances in fish and other meat products in particular will combine with nitrite and when fed to rats result in alterations in gastric epithelium suggestive of neoplastic changes.

Vitamin C and Stomach Cancer

The question of whether or not vitamin C (ascorbic acid) has an effect on cancer of the stomach is controversial. It has been shown to protect against cancer (36), to have no effect (37), and to possibly enhance carcinogenesis (38). There are, however, data which, when put in perspective, seem to indicate that moderate doses of ascorbic acid offer some protection against experimental gastric cancer associated with carcinogens or procarcinogens in foods. Human epidemiologic data also indicate that ascorbic acid is negatively associated with the development of gastric cancer (39-41).

There is no convincing evidence that vitamin C is useful as a therapeutic agent in cancer patients even though there is a great deal of enthusiasm for such treatment (42-46).

We have observed in our laboratory (26) that tumors of the forestomach were inhibited by high levels of vitamin A (retinyl acetate, RA). Hamsters given carcinogenic doses of benz(a)pyrene and then treated with either 1600 or 2400 μg of retinol per week had a significantly decreased number of tumors (Table 8). Since the vitamin A was acting during the post exposure period it was effective on the

Table 7. Incidence of stomach epithelium hyperplasia and intestinal metaplasia.^a

Group and treatment	Effective no. of rats	Forestomach squamous cell hyperplasia	Glandular stomach	
			Glandular hyperplasia	Intestinal metaplasia
I Fish extract alone	8	0	1	1
II Fish extract + NaNO_2	12	5	6	6

^a Data of Weisburger et al. (35).

Table 8. Incidence of forestomach papillomas induced by BP in hamsters and effects of retinyl acetate (RA).

RA dose, μg wk	No. of hamsters	% with papilloma	No. of papillomas/papilloma-bearing hamster ^a
100	109	50	2.9 ± 0.2
1600	111	25 ^b	2.2 ± 0.3
2400	102	26 ^b	3.1 ± 0.2

^a Means \pm S.E.

^b Compared to hamsters given 100 μg RA/week, $p < 0.005$.

promotional stage of tumor development, much like the effect of retinoids on the mouse two-stage skin model.

While the incidence of gastric cancer has steadily decreased in the United States over the past decades, nothing factual is known about the reasons for the decline. It has been proposed that the decrease is because of better distribution of food with adequate fresh food sources of vitamins C and E. Furthermore, there has been an increase in the use of antioxidants in foods of natural or synthetic origin; this may have had some effect on the decline but other suggestions are equally viable. For example, another suggestion has been that foods are now refrigerated; the improvement in preserving of foods during the past three or four decades is believed to contribute to the decrease in gastric carcinoma during this period. This however conflicts with the observations of Wynder et al. (47) that gastric cancer exhibits a north-south gradient (or south-north gradient in the Southern Hemisphere) with greater incidence in the more temperate or frigid zones.

Liver Carcinogenesis

The current literature includes many papers supporting the development of cancer of the liver through a multistep process involving initiation and promotion (48-49). However, despite the large numbers of reports and the clear indications that liver carcinogenesis is a complex multistage process,

very little is known about the influence of diet on such processes. Reports in the literature use the terms initiation and promotion in a rather loose manner.

Our laboratory has done much of the work on the effects of lipotrope deficiency on hepatocarcinogenesis (50-54). While it has been clearly shown that lipotrope deficiency profoundly affects the rate and incidence of liver tumors, the time during which its effect is exerted has not been clearly defined. A few examples are given below to illustrate the effects of lipotrope deficiency on the enhancement of hepatocellular carcinogenesis, some of which indicate an effect during both initiation and promotion stages. Table 9 lists a few of the experiments with various types of carcinogens showing the effects of a low lipotrope diet on the development of tumors. Clearly, except for one carcinogen (dimethylnitrosamine, DMN), there is an enhancing effect of the diet on tumorigenesis. Since these were fed or administered during and after the carcinogen we are unable at this point to determine whether or not the effect was during initiation or promotion or both. It appears that a part of the effect of the lipotrope deficient diet on hepatocarcinogenesis is exerted (55) through its influence on microsomal enzyme activity (Table 10) and on aflatoxin metabolism (Table 11).

Table 12 presents data that indicate the dietary effects of quality and quantity of two types of fat on aflatoxin carcinogenesis. From these data it would appear that beef fat exerted just as much effect in the promotional or growth stage of the tumors as in

Table 9. Hepatocarcinogenesis in rats fed diets deprived of lipotropes.^a

Treatment	Tumor incidence, %	
	Control	Lipotrope-deficient
AFB ₁ , 375 µg (total 3 experiments)	6-15	22-87
Diethylnitrosamine, 40 mg/kg, 18 weeks	70	88
Dibutylnitrosamine, 3.8 mg/kg total	24	64
Dimethylnitrosamine		
100 mg/kg in diet, 3 weeks	28	27
25-50 mg/kg in diet, 8 weeks	74	50
Acetylaminofluorene, 0.0125% in diet, 16 weeks 2 experiments	19-61	41-91
DDCP, 195 mg total	29	63

^a Data of Rogers and Newberne (54).

Table 10. Dietary effects on AFB₁ metabolism.^a

	Control	Lipotrope-deficient
AFB ₁ metabolism		
AFB ₁	45.6 ± 3.8	43.5 ± 4.1
Aflatoxin Q ₁	0.76 ± 0.04	0.84 ± 0.34
Aflatoxin M ₁	0.72 ± 0.04	0.88 ± 0.12
MFO type 1 spectra		
ΔA _{max} , mmole/mg protein	10.3 ± 0.8	7.6 ± 0.9
K _s , mM	0.27 ± 0.04	0.38 ± 0.10

^a Data of Campbell et al. (55).

promotion and initiation stages combined. This was not the case with corn oil, since with corn oil only, after the carcinogen has been given, there was significantly less effect than when it was given during and after carcinogen exposure (initiation and promotion stages).

Another most interesting promoting effect has recently been revealed in our laboratory with the $B_6C_3F_1$ mouse which has a high incidence of spontaneous liver tumors (57). Table 13 shows that a simple partial hepatectomy significantly enhances the tumor incidence to 40% compared to zero in the control. Choline deficiency has an even more profound effect raising the level to 100%. Interestingly, the deficient diet plus a partial hepatectomy appeared to be less tumor enhancing than the choline deficiency alone. This observation will be reported in more detail in another publication.

Pancreatic Cancer

Carcinoma of the pancreas is a common, highly lethal gastrointestinal type of malignancy. The incidence appears to be rising in United States populations and now is second only to cancer of the colon

as a cause of death from gastrointestinal neoplasms. Each year from 20,000 to 25,000 new cases are diagnosed in the United States, and 95% of these are expected to die from the disease. As with other types of cancer, particularly esophagus, excessive alcohol, smoking, and exposure to industrial compounds including benzidine, urea, and methylnitrosamine appear to play a prominent role in cancer of the pancreas. However, proof of a cause-effect relationship has not been firmly established.

A number of epidemiologic studies, including the recent controversial report by McMahon, has served to increase the interest in pancreatic carcinoma (58, 59). While the epidemiologic data are not firm, experimental results from studies conducted in experimental animals are convincing. The induction of pancreatic cancer in rats by the injection of azaserine was first recorded in 1975 (60). This coincided with the reports of several other animal models for the induction of pancreatic cancer (61). Some of the most interesting experimental work has come from the laboratory of Longnecker, who showed that pancreatic carcinogenesis is enhanced in animals fed diets containing 20% corn oil (Table 14).

Additional data from Longnecker's laboratory

Table 11. Marginal lipotrope deficiency and microsomal characteristics.^a

	Control	Lipotrope-deficient
Microsomal protein, mg/g	24.4 ± 1.5	18.7 ± 1.1
Cytochrome P-450, nmole/mg protein	1.8 ± 0.1	0.5 ± 0.1
Ethylmorphine <i>N</i> -demethylase nmole/mg protein	787 ± 79	572 ± 40
nmole/100 g body weight × 10 ⁴	6.0	4.6
Ethoxycoumarin <i>O</i> -dealkylase nmole/mg protein	0.350 ± 0.09	0.215 ± 0.03
nmole/100 g body weight	26.8	17.3
Cytochrome c reductase nmole/mg protein	66.2 ± 3.7	52.4 ± 3.5
nmole/100 g body weight × 10 ²	50.8	42.2

^aData of Campbell et al. (55)

Table 13. Promoting effect of choline deficiency and partial hepatectomy on spontaneous $B_6C_3F_1$ mouse hepatocarcinogenesis.

Treatment	Tumors after 9 mo., %
Control	0.0
Control + partial hepatectomy	20.0
Choline-deficient	100.0
Choline-deficient + partial hepatectomy	40.0

Data of Newberne, de Camargo and Clark (57).

Table 14. Effect of dietary fat on pancreatic cancer in rats.

Treatment	Pancreas weight, g	Pancreatic tumors
Control	1.4 ± 0.3	0.0
Control + azaserine	2.6 ± 1.4	71.0
Unsaturated fat + azaserine	4.7 ± 2.5	100.0
Saturated fat + azaserine	2.6 ± 0.8	75.0

^aData of Roebuck et al. (62).

Table 12. Effects of dietary fat during and after exposure to a carcinogenic dose of AFB₁ on tumor incidence.^a

Beef fat		Corn oil		Incidence of liver tumors after 14 mo.		Incidence of lung metastases, %
During treatment with AFB ₁	After treatment with AFB ₁	During treatment with AFB ₁	After treatment with AFB ₁	No.	%	
+	+	0	0	32	53	25.0
0	+	0	0	28	51	21.0
0	0	+	+	60	100	60.3
0	0	0	+	38	66	36.8

^aData of Newberne et al. (56).

have shown that rats fed a semipurified diet developed more pancreatic tumors than rats fed a commercial laboratory chow. Furthermore, selected feeding of these diets during the administration of carcinogen and after the carcinogen had been discontinued seemed to indicate that the inhibitory effect of the chow diet on carcinogenesis was exerted during the post-initiation phase (Table 15). In addition, the supplementation of the diet with retinyl acetate during the post initiation phase also inhibited the progression of the pancreatic tumors induced by azaserine (Table 16). These studies then seem to infer that the influence of dietary fat quality, and perhaps quantity, as well as vitamin A are acting at the post initiation phase of tumorigenesis, (63).

A number of other investigators have studied the effects of diet on pancreatic tumors but there is little information as to whether or not the diets act at the initiation or at the post-initiation (promotion) stages of tumor development (61, 64). The summation of available data indicate that diets high in unsaturated fat enhance pancreatic carcinogenesis in the rat animal model.

Colon Carcinogenesis

There have been numerous studies on the epidemiology of colon cancer in recent years, in particular, on how dietary factors may influence this type of neoplasm (65-69). While some of these studies

suggest that diets high in fat and low in fiber may be associated with increased incidence of large bowel cancer in human populations, the data are not so clearly defined. However, it is apparent that the more economically developed societies have a greater incidence of colon cancer with the highest incidence rates found in North America, New Zealand and Western Europe. Intermediate rates are found in Eastern Europe and the Balkans and the lowest incidences are observed in Africa, Latin America and Asia, (66, 70).

The evidence for environmental dietary factors and colon cancer is particularly convincing from studies of migrants from Japan and Poland to the United States and Australia (71, 72). These studies indicate that colon cancer rates are higher in the first and second generation Japanese immigrants to the United States and in Polish immigrants to Australia than in native Japanese in Japan and in native Poles from Poland. The increasing westernization of the Japanese diet coincides with the increased development of colon cancer in the Japanese.

In the United States, the incidence of colon cancer in Mormons is lower than in other U.S. population groups with the exception of the Seventh Day Adventists (73). Mormons consume more breads made from whole grain and more vegetables and fruits than their counterparts, who have a higher incidence of colon cancer, (68). Furthermore, a number of other studies have shown that those who consume less meat and more vegetables have smaller risk of colon cancer (74, 75).

A number of investigators have suggested that dietary fat is closely correlated with the incidence of colon cancer. While much of the data are interesting and suggestive, even the experimental results in animal models require validation (65, 76, 77). Others have suggested that the large bowel cancer rates are closely correlated with the consumption of meat and other animal proteins as well as total fat (78). While some of the data are convincing on the face of the evidence, other reports leave the matter open to question; additional well designed epidemiologic and experimental animal studies are required. The following few cases illustrate one view of the current state of knowledge.

Burkitt (79) was one of the first to suggest that the low incidence of bowel cancer in most African populations was associated with a high dietary fiber intake. Conversely, low fiber diets consumed by Western populations are associated with a high incidence of colon cancer. Further evidence from a recent study compares Finland and Denmark populations with New York populations. Some Finnish populations are at low risk, whereas Danish and

Table 15. Effect of diet on postinitiation phase of azaserine-induced pancreatic tumors.^a

Diet/treatment		Pancreas weight, g ^b	Mean no. nodules/pancreas ^b
Initiation phase	Post-initiation phase		
Control, semisynthetic	Semisynthetic	1.3 ± 0.2	0.7 ± 0.9
Semisynthetic + AZA	Semisynthetic	1.5 ± 0.2	16.7 ± 7.6
Chow + AZA	Semisynthetic	1.4 ± 0.1	14.3 ± 11.7

^aData of Longnecker et al. (63).

^bMeans, 10 rats per group.

Table 16. Effect of diet on postinitiation phase of azaserine carcinogenesis in rats.^a

Diet/treatment		Body weight, g	Rats with pancreatic tumors	
Initiation phase	Post-initiation phase		No.	%
Control, semisynthetic	Semisynthetic	745 ± 105	0/16	0
Semisynthetic + AZA	Semisynthetic	706 ± 83	10/18	56
Semisynthetic + AZA	Chow	578 ± 55	0/17	0
Semisynthetic + AZA	Semisynthetic + retinyl acetate	717 ± 77	3/30	30

^aData of Longnecker et al. (63).

New York populations are at high risk; the indications from the study were that the low risk of colon cancer in Finland was associated with high dietary fiber intake (80, 81).

In addition to the fat and fiber concepts, other investigators have suggested that bile acid secretion associated with fat content in the diet is related in some way to colon cancer. That aspect will be covered in more detail by others at this conference. It must be borne in mind, however, that this is an extremely complex area and that it is quite unlikely that any one single dietary nutrient is going to have a primary effect.

Experimental studies in animal models have helped to clarify some aspects of dietary effects on colon cancer. The model using dimethylhydrazine (DMH)-treated rats has been the most widely used, and while there has been a suggestion that DMH produces intestinal tumors by way of two-stage mechanism (82), this is not yet firmly established.

If such a two-stage mechanism could be clearly shown for colon carcinogenesis, it would make it much simpler to separate the initiation stages from the post initiation (promotion) stages of tumor development. It is pointed out by some investigators (83, 84) that, while these suggestions are provocative and interesting, the entire process appears to be much too complex at this point to clearly separate initiation from promotion. Among other factors, the necessity for multidose scheduling in order to produce the tumors complicates separation into distinct stages. However, separation of the initiation from promotional stages should be a primary goal of investigators.

Many investigators have studied the role of dietary fat in colon carcinogenesis as well as the effects of fiber and other factors. At this point if there are specific factors involved in promotion of colon cancer it seems to be associated more with dietary fat. For example, Nigro et al. (85) compared tumors in rats by administration of a chemical carcinogen and observed that 35% fat had an enhancing effect compared to lower fat levels.

In studies conducted in our laboratories diets marginally deficient in lipotropes but high in fat appear to enhance DMH induced colon carcinogenesis in rats (86). While these data suggest that the total amount of dietary fat may play a role in the pathogenesis of colon cancer it does not indicate the time-span during carcinogenesis that the fat exerts its effect. Table 17 lists the results of studies, typical of many other investigations that we have conducted in our laboratory.

Typical also of other studies are those of Reddy et al. (87) which indicated that animals fed 20% lard or 20% corn oil were more susceptible to colon tu-

mor induction by DMH than those fed 5% of either of these two fats. Further, Broitman et al. (88) showed that rats fed a 25% safflower oil diet and treated with DMH had more large bowel tumors than animals fed either the 5% or 20% coconut oil diets. These data indicate that diets rich in polyunsaturated fats are more effective tumor promoters than diets containing saturated fat at a comparable level.

Recent studies by Bull et al. (89) have indicated that the enhanced tumorigenesis in animals fed high fat diets is due to promotional effects. The ingestion of high fat diets increased the incidence of tumors induced by azoxymethane if fed after azoxymethane administration but not during or before treatment with carcinogen. This indicates an example of probable tumor promotion by dietary fat and emphasizes further this encouraging area for research. It is more significant since some characteristics in human tumors correspond well with those in a variety of animal models (90).

Another dietary constituent that is in some way related to enhancement of colon carcinogenesis is vitamin A and the retinoids, (25, 50). In the case of the retinoids, however, it appears that a deficiency enhances the induction of tumors. Using the colon carcinogen DMH we have clearly shown that tumor induction is accelerated by vitamin A deficiency. Another example, using a different type of carcinogen, illustrates the apparent enhancing effect of a deficiency of vitamin A on colon carcinogenesis but does not clearly point out the period during which the effect is exerted in the aflatoxin B₁ model where not only liver but colon tumors are produced (Table 18). An interesting and perhaps significant observation is that in the vitamin A-deficient colon there is a modification of microsomal enzyme activity and of aflatoxin metabolism, and binding to cellular macromolecules of the colon epithelium (91). In this case, the effects might be exerted either during exposure to the carcinogen or following exposure, since removal of the DNA adducts can continue over a long period of time following exposure to the carcinogen. Thus if the rate of removal or repair of DNA were affected during the promotional stage, this could have an influence directly on carcinogenesis.

Table 17. Dietary fat and rat colon tumors.^a

Diet	Dimethylhydrazine dose, mg/kg	Rats with colon carcinoma, %	No. of tumors/tumor-bearing rat
Control	300	86	2.0
High-fat	300	100	3.7
Control	150	56	1.1
High-fat	150	85	2.6

^aTumors were induced by dimethylhydrazine.

Table 18. Enhancement of AFB₁ colon carcinogenesis by Vitamin A deficiency.^a

Vitamin A intake	Sex	Tumor incidence, %	
		Colon	Liver
Adequate	M	0	48
Deficient	M	12	22
Excessive	M	0	38
Adequate	M	4	88
	F	8	79
Deficient	M	29	89
	F	28	76
Excessive	M	8	92
	F	10	84

^aData of Newberne and Rogers (50).

Discussion and Conclusions

As pointed out earlier in this presentation, there are a number of chemicals recognized as initiators and many others that are recognized as promoters of the carcinogenic process. It is quite important that we understand not only the mechanisms of initiation of carcinogenesis but the later events that occur in the putative promotional stage. A good example of the complexity of the carcinogenic process is exhibited by benzo(a)pyrene. When an animal is exposed to this chemical there is no immediate induction of neoplasia. Generally, there is a lag of several months, and then a stepwise change in the properties of cells occurs which usually includes hyperplasia, followed by a benign tumor and later, a fully malignant one. It is the latter steps, from a clinical point of view, which are the most important and troublesome because if they did not occur as has been repeatedly shown, benign tumors could be removed and the disease could be cured. It appears that early stages of a number of target organ cancers may be reversible or they can regress. The latter stages are much less readily reversed and it is the later stages which provide the greatest challenge to investigators.

Using benzo(a)pyrene as an example, it is generally accepted that the first step in benzo(a)pyrene carcinogenesis is the covalent binding of the carcinogen to the nucleic acids in the cells. Before this binding can occur, the carcinogen must be activated, in this case, to a dihydrodiolepoxide which in turn links by way of the number 10 position to the 2-amino of guanine. Thus the metabolism of the compound and factors that influence this can be of major importance to appearance of cancer (3). It is during this period that dietary factors may act.

In our attempts to learn more about promotion specifically, the surface has only been scratched; we know very little about nutrients which affect promotion and virtually nothing about the mechanisms

whereby this is achieved. In one sense, considering the effects of vitamin A, it would appear that differentiation is the key to the whole process. Since it appears that vitamin A and the retinoids act during the promotional stage, differentiation must be the key event that prevents the formation of malignant tumors. However to produce a cancer cell, the previous pattern of gene expression of that cell must be reprogrammed. Perhaps it is during this period that some of the dietary effects are exerted. To convert a cell from one that is normally differentiated it must be diverted from the usual mode of terminal differentiation and converted to one of exponential division that proceeds into the carcinogenic process, eventuating in cancer. These aspects we know very little about even in the well defined mouse skin two-stage carcinogenic process.

The most we can say at this point about nutrients and promotion of intestinal tumors is that there does appear to be some relationship between promoters and gastrointestinal cancer. It appears also, at this point, that it is premature to attempt to assign specific carcinogens to stages of initiation or promotion; the process is probably much more complex. Furthermore, it seems illogical to assume that nutrients act only during one period of tumor induction. Since, under some circumstances, initiators are mutagenic and promoters are not, definitions should be worked out to encompass the more complex areas related to tumor initiation and promotion, particularly with diet and nutrition.

In terms of gastrointestinal cancer we can say that dietary ingredients do affect cancer of the esophagus, the stomach, the pancreas, the liver and the colon. However, in most cases it is not clear as to whether it is during the initiation period or the promotional stage that these ingredients are exerting their effects. This area of research has some significant and far-reaching implications in attempts to prevent cancer.

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